

Amendments to the Specification:

The following paragraph replaces the paragraph at page 8, lines 19-27:

“Isolated nucleic acid molecules that encode *Renilla reniformis* fluorescent proteins are provided. Nucleic acid probes and primers derived therefrom are also provided. Functionally equivalent nucleic acids, such as those that hybridize under conditions of high stringency to the disclosed molecules and those that have high sequence identity, are also contemplated. Nucleic acid molecules and the encoded proteins are set forth in SEQ ID. NOs. 23-27, an exemplary mutin is set forth in SEQ ID. NO. 33. Also contemplated are nucleic acid molecules that encode the protein set forth in SEQ ID. NO. 27.”

The following paragraphs replace the paragraphs at page 11, lines 7-27:

“Isolated nucleic acids that encode GFP from *Renilla reniformis* are provided herein. Also provided are isolated and purified nucleic acids that encode a component of the bioluminescence generating system and the green fluorescent protein (GFP) (see SEQ ID NOs. 23-27). In particular, nucleic acid molecules that encode *Renilla reniformis* green fluorescent protein (GFPs) and nucleic acid probes and primers derived therefrom are provided (see SEQ ID NOs. 23-26).

Nucleic acid probes and primers containing 14, 16, 30, 100 or more contiguous nucleotides from any of SEQ ID NOs. 23-26 are provided. Nucleic acid probes can be labeled, if needed, for detection, containing at least about 14, preferably at least about 16, or, if desired, 20 or 30 or more, contiguous nucleotides of the sequence of nucleotides encoding the *Renilla reniformis* GFP.”

The following paragraph replaces the paragraph at page 13, lines 11 through page 14, line 4:

“The nucleic acids provide an opportunity to produce luciferases and GFPs, which have advantageous application in all areas in which luciferase/luciferins and GFPs have application. The nucleic acids can be used to obtain and produce GFPs and GFPs from other, particularly *Renilla*, species using the probes described herein that correspond to conserved regions. These

GFPs provide a means to amplify the output signal of bioluminescence generating systems. Renilla GFP has a single excitation absorbance peak in blue light (and around 498 nm) and a predominantly single emission peak around 510 nm (with a small shoulder near 540 nm). This spectrum provides a means for it to absorb blue light and efficiently convert it to green light. This results in an amplification of the output. When used in conjunction with a bioluminescence generating system that yields blue light, such as *Aequorea* or *Renilla* or *Vargula* (*Cypridina*), the output signal for any application, including diagnostic applications, is amplified. In addition, this green light can serve as an energy donor in fluorescence-based assays, such as fluorescence polarized assays, FET (fluorescent energy transfer) assays, FRET (fluorescent resonance energy transfer) assays and HTRF (homogeneous time-resolved fluorescence) assays. Particular assays, herein referred to as BRET (bioluminescence resonance energy transfer assays in which energy is transferred from a bioluminescence reaction of a luciferase to a fluorescent protein), are provided.

The following paragraph replaces the paragraph at page 14, line 21 through page 15, line 5:

“The DNA may be introduced as a linear DNA molecule (fragment) or may be included in an expression vector for stable or transient expression of the encoding DNA. In certain embodiments, the cells that contain DNA or RNA encoding a *Renilla* GFP also express the recombinant *Renilla* GFP or polypeptide. It is preferred that the cells selected to express functional GFPs that retain the ability to fluorescence and that are not toxic to the host cell. In some embodiments, cells may also include heterologous nucleic acid encoding a component of a bioluminescence-generating system, preferably a photoprotein or luciferase. In preferred embodiments, the nucleic acid encoding the bioluminescence-generating system component is isolated from the species *Aequorea*, *Vargula*, *Pleuromamma*, *Ptilosarcus* or *Renilla*. In more preferred embodiments, the bioluminescence-generating system component is a *Renilla reniformis* luciferase or *mulleri* including the amino acid sequence set forth in SEQ ID NO. 19 or the *Pleuromamma* luciferase set forth in SEQ ID NO. 28, or the *Gaussia* luciferase set forth in SEQ ID NO. 19.”

The following paragraph replaces the paragraph on page 24, lines 16-21:

“FIGURE 4 depicts the substitution of altered fluorophores into the background of *Ptilosarcus*, *Renilla mulleri* and *Renilla reniformis* GFPs (the underlined regions correspond to amino acids 56-75 of SEQ ID NO. 27 *Renilla reniformis* GFP; amino acids 59-78 of SEQ ID NO. 16 *Renilla mulleri* GFP; and amino acids 59-78 of SEQ ID NO. 32 for *Ptilosarcus* GFP).”

The following paragraph replaces the paragraph on page 27, lines 13-19:

“As used herein, luminescence refers to the detectable electromagnetic (EM) radiation, generally, ultraviolet (UV), infrared (IR) or visible EM radiation that is produced when the excited product of an exergic chemical process reverts to its ground state with the emission of light. Chemiluminescence is luminescence that results from a chemical reaction. Bioluminescence is chemiluminescence that results from a chemical reaction using biological molecules (or synthetic versions or analogs thereof) as substrates and/or enzymes.

The following paragraph replaces the paragraph on page 30, lines 4-12:

“As used herein, a *Renilla reniformis* green fluorescent protein (GFP) refers to a fluorescent protein that is encoded by a sequence of nucleotides that encodes the protein of SEQ ID NO. 27 or to a green fluorescent protein from *Renilla reniformis* having at least 80%, 90% or 95% or greater sequence identity thereto; or that is encoded by a sequence of nucleotides that hybridizes under high stringency along its full length to the coding portion of the sequence of nucleotides set forth in any of SEQ ID NOs. 23-25. A *Renilla reniformis* GFP is protein that is fluorescent and is produced in a *Renilla reniformis*.”

The following paragraph replaces the paragraph on page 34, line 25 through page 35, line 5:

“As used herein, a nucleic acid probe is single-stranded DNA or RNA that has a sequence of nucleotides that includes at least 14 contiguous bases, preferably at least 16 contiguous bases, typically about 30, that are the same as (or the complement of) any 14 or more contiguous bases set forth in any of SEQ ID NOs. 23-25 and herein. Among the preferred regions from which

to construct probes include 5' and/or 3' coding sequences, sequences predicted to encode regions that are conserved among *Renilla* species GFPs are for isolating GFP-encoding nucleic acid from *Renilla* libraries.”

The following paragraphs replace the paragraphs on page 49, lines 6-24:

“Purified *Renilla reniformis* GFP and muteins thereof are provided. Presently preferred *Renilla* GFP for use in the compositions herein is *Renilla reniformis* GFP having the sequence of amino acids set forth in SEQ ID NO. 27. The *Renilla* GFP and GFP peptides can be isolated from natural sources or isolated from a prokaryotic or eukaryotic cell transfected with nucleic acid that encodes the *Renilla* GFP and/or GFP peptides, such as those encoded by the sequences of nucleotides set forth in SEQ ID NOS. 23-25.

The encoding nucleic acid molecules are provided. Preferred are those that encode the protein having the sequence of amino acids (SEQ ID NO. 27):

mdlaklgkvevmptkinleglvgdhafsmevgvgegnilegtqevkisvtkgaplpfafdivsv
afsygnraytgypeeisdyflqsfpegftyerniryqdggtaivkdisledgkfivnvdfkakdl
rrmgpvmqqdivgmqpsyesmytnvtsvigeciiafklqtgkhfthyhmrtvykskpvvet
mplyhfiqhrlvktnvdtasgyvvqhetaiaahstikkiegsip,

and is preferably the sequence set forth in SEQ ID NO. 26.

In particular, nucleic acid molecules encoding a *Renilla reniformis* GFP having any of the following sequences are provided (see SEQ ID NOS. 23-25):”

The following paragraph replaces the paragraph on page 51, lines 7-8:

“An exemplary mutein is set forth in SEQ ID NO. 33, and humanized codon are set forth in SEQ ID NO. 26.”

The following paragraph replaces the paragraph on page 55, lines 24-31:

“Thus, in some embodiments, a crude extract or merely grinding up the organism may be adequate. Generally, however, substantially pure components are used. Also, components may be synthetic components that are not isolated from natural sources. DNA encoding luciferases

is available (see, e.g., SEQ ID NOs. 1-13) and synthetic and alternative substrates have been devised. The DNA listed herein is only representative of the DNA encoding luciferases that are available.”

The following paragraph replaces the paragraph on page 57, line 30 to page 58, line 10:

“Examples of luciferases include, but are not limited to, those isolated from the ctenophores *Mnemiopsis* (mnemiopsin) and *Beroe ovata* (berovin), those isolated from the coelenterates *Aequorea* (aequorin), *Obelia* (obelin), *Pelagia*, the *Renilla* luciferase, the luciferases isolated from the mollusca *Pholas* (pholasin), the luciferases isolated from fish, such as *Aristostomias*, *Pachystomias* and *Porichthys* and from the ostracods, such as *Cyprindina* (also referred to as *Vargula*). Preferred luciferases for use herein are the Aequorin protein, *Renilla* luciferase and *Cypridina* (also called *Vargula*) luciferase (see, e.g., SEQ ID NOs. 1, 2, and 4-13). Also, preferred are luciferases which react to produce red and/or near infrared light. These include luciferases found in species of *Aristostomias*, such as *A. scintillans*, *Pachystomias*, *Malacosteus*, such as *M. niger*.”

The following paragraph replaces the paragraph on page 62, line 14, to page 63, line 2:

“*Renilla* luciferases, DNA encoding *Renilla reniformis* luciferase, and use of the *Renilla reniformis* DNA to produce recombinant luciferase, as well as DNA encoding luciferase from other coelenterates, are well known and available (see, e.g., SEQ ID NO. 1, U.S. Patent Nos. 5,418,155 and 5,292,658; see, also, Prasher *et al.* (1985) *Biochem. Biophys. Res. Commun.* 126:1259-1268; Cormier (1981) “*Renilla* and *Aequorea* bioluminescence” in *Bioluminescence and Chemiluminescence*, pp. 225-233; Charbonneau *et al.* (1979) *J. Biol. Chem.* 254:769-780; Ward *et al.* (1979) *J. Biol. Chem.* 254:781-788; Lorenz *et al.* (1981) *Proc. Natl. Acad. Sci. U.S.A.* 88:4438-4442; Hori *et al.* (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74:4285-4287; Hori *et al.* (1975) *Biochemistry* 14:2371-2376; Hori *et al.* (1977) *Proc. Natl. Acad. Sci. U.S.A.* 74:4285-4287; Inouye *et al.* (1975) *Jap. Soc. Chem. Lett.* 141-144; and Matthews *et al.* (1979) *Biochemistry* 16:85-91. The DNA

encoding *Renilla reniformis* luciferase and host cells containing such DNA provide a convenient means for producing large quantities of *Renilla reniformis* enzyme, such as in those known to those of skill in the art (see, e.g., U.S. Patent Nos. 5,418,155 and 5,292,658, which describe recombinant production of *Renilla reniformis* luciferase)."

The following paragraph replaces the paragraph on page 64, line 17, to page 65, line 15:

"The aequorin system is well known (see, e.g., Tsuji *et al.* (1986) "Site-specific mutagenesis of the calcium-binding photoprotein aequorin," *Proc. Natl. Acad. Sci. USA* 83:8107-8111; Prasher *et al.* (1985) "Cloning and Expression of the cDNA Coding for Aequorin, a Bioluminescent Calcium-Binding Protein," *Biochemical and Biophysical Research Communications* 126:1259-1268; Prasher *et al.* (1986) *Methods in Enzymology* 133:288-297; Prasher, *et al.* (1987) "Sequence Comparisons of cDNAs Encoding for Aequorin Isoforms," *Biochemistry* 26:1326-1332; Charbonneau *et al.* (1985) "Amino Acid Sequence of the Calcium-Dependent Photoprotein Aequorin," *Biochemistry* 24:6762-6771; Shimomura *et al.* (1981) "Resistivity to denaturation of the apoprotein of aequorin and reconstitution of the luminescent photoprotein from the partially denatured apoprotein," *Biochem. J.* 199:825-828; Inouye *et al.* (1989) *J. Biochem.* 105:473-477; Inouye *et al.* (1986) "Expression of Apoequorin Complementary DNA in *Escherichia coli*," *Biochemistry* 25:8425-8429; Inouye *et al.* (1985) "Cloning and sequence analysis of cDNA for the luminescent protein aequorin," *Proc. Natl. Acad. Sci USA* 82:3154-3158; Prendergast *et al.* (1978) "Chemical and Physical Properties of Aequorin and the Green Fluorescent Protein Isolated from *Aequorea forskalea*" *J. Am. Chem. Soc.* 100:3448-3453; European Patent Application 0 540 064 A1; European Patent Application 0 226 979 A2; European Patent Application 0 245 093 A1 and European Patent Application 0 245 093 B1; U.S. Patent No. 5,093,240; U.S. Patent No. 5,360,728; U.S. Patent No. 5,139,937; U.S. Patent No. 5,422,266; U.S. Patent No. 5,023,181; U.S. Patent No. 5,162,227; and SEQ ID NOs. 5-13, which set forth DNA encoding the apoprotein; and a form, described in U.S. Patent No. 5,162,227, European Patent Application 0 540 064 A1 and Sealite Sciences Technical Report No. 3 (1994), is commercially available from Sealite, Sciences, Bogart, GA as AQUALITE®."

The following paragraph replaces the paragraph on page 66, lines 16-21:

“Naturally-occurring apoequorin is not a single compound but rather is a mixture of microheterogeneous molecular species. *Aequoria* jellyfish extracts contain as many as twelve distinct variants of the protein (see, e.g., Prasher et al. (187) *Biochemistry* 26:1326-1332; Blinks *et al.* (1975) *Fed. Proc.* 34:474). DNA encoding numerous forms has been isolated (see, e.g., SEQ ID NOs. 5-9 and 13).”

The following paragraphs replace the paragraphs on page 67, line 18 to page 69, line 6:

“Numerous isoforms of the aequorin apoprotein have been identified and isolated. DNA encoding these proteins has been cloned, and the proteins and modified forms thereof have been produced using suitable host cells (see, e.g., U.S. Patent Nos. 5,162,227, 5,360,728, 6,093,240; see, also, Prasher *et al.* (1985) *Biophys. Biochem. Res. Commun.* 126:1259-1268; Inouye et al. (1986) *Biochemistry* 25:8425-8429). U.S. Patent No. 5,093,240; U.S. Patent No. 5,360,728; U.S. Patent No. 5,139,937; U.S. Patent No. 5,288,623; U.S. Patent No. 5,422,266; U.S. Patent No. 5,162,227 and SEQ ID NOs. 5-13, which set forth DNA encoding the apoprotein; and a form is commercially available from Sealite, Sciences, Bogart, GA as AQUALITE®). DNA encoding apoequorin or variants thereof is useful for recombinant production of high quantities of the apoprotein. The photoprotein is reconstituted upon addition of the luciferin, coelenterazine, preferably a sulfated derivative thereof, or an analog thereof, and molecular oxygen (see, e.g., U.S. Patent No. 5,023,181). The apoprotein and other constituents of the photoprotein and bioluminescence generating reaction can be mixed under appropriate conditions to regenerate the photoprotein and concomitantly have the photoprotein produce light. Reconstitution requires the presence of a reducing agent, such as mercaptoethanol, except for modified forms, discussed below, that are designed so that a reducing agent is not required (see, e.g., U.S. Patent No. 5,093,240).

For use herein, it is preferred aequorin is produced using DNA, such as that set forth in SEQ ID NOs. 5-13 and known to those of skill in the art or modified forms thereof. The DNA encoding aequorin is expressed in a host cell, such as *E. coli*, isolated and reconstituted to produce

the photoprotein (see, e.g., U.S. Patent Nos. 5,418,155, 5,292,658, 5,360,728, 5,422,266, 5,162,227).

Of interest herein, are forms of the apoprotein that have been modified so that the bioluminescent activity is greater than unmodified apoaequorin (see, e.g., U.S. Patent No. 5,360,728, SEQ ID NOs. 10-12). Modified forms that exhibit greater bioluminescent activity than unmodified apoaequorin include proteins including sequences set forth in SEQ ID NOs. 10-12, in which aspartate 124 is changed to serine, glutamate 135 is changed to serine, and glycine 129 is changed to alanine, respectively. Other modified forms with increased bioluminescence are also available.

For use in certain embodiments herein, the apoprotein and other components of the aequorin bioluminescence generating system are packaged or provided as a mixture, which, when desired is subjected to conditions under which the photoprotein reconstitutes from the apoprotein, luciferin and oxygen (see, e.g., U.S. Patent No. 5,023,181; and U.S. Patent No. 5,093,240). Particularly preferred are forms of the apoprotein that do not require a reducing agent, such as 2-mercapto-ethanol, for reconstitution. These forms, described, for example in U.S. Patent No. 5,093,240 (see, also Tsuji *et al.* (1986) *Proc. Natl. Acad. Sci. U.S.A.* 83:8107-8111), are modified by replacement of one or more, preferably all three cysteine residues with, for example serine. Replacement may be effected by modification of the DNA encoding the aequorin apoprotein, such as that set forth in SEQ ID NO. 5, and replacing the cysteine codons with serine.”

The following paragraph replaces the paragraph at page 75, lines 19-27:

“DNA clones encoding luciferases from various insects and the use to produce the encoded luciferase is well known. For example, DNA clones that encode luciferase from *Photinus pyralis*, *luciola cruciata* (see, e.g., de West *et al.* (1985) *Proc. Natl. Acad. Sci. U.S.A.* 82:7870-7873; de West *et al.* (1986) *Methods in Enzymology* 133:3; U.S. Patent No. 4,968,613, see, also SEQ ID NO. 3) are available. The DNA has also been expressed in *Saccharomyces* (see, e.g., Japanese Application No. JP 63317079, published December 26, 1988, KIKKOMAN CORP) and in tobacco.”

The following paragraph replaces the paragraph on page 76, lines 6-18:

“Modified luciferases can generate light at different wavelengths (compared with native luciferase), and thus, may be selected for their color-producing characteristics. For example, synthetic mutant beetle luciferase(s) and DNA encoding such luciferases that produce bioluminescence at a wavelength from wild-type luciferase are known (Promega Corp, International PCT Application No. WO 95/18853, which is based on U.S. application Serial No. 08/177,081. The mutant beetle luciferase has an amino acid sequence differing from that of the corresponding wild-type *Luciola cruciata* (see, e.g., U.S. Patent Nos. 5,182,202, 5,219,737, 5,352,598, see, also SEQ ID NO. 3) by a substitution(s) at one or two positions. The mutant luciferase produces a bioluminescence with a wavelength of peak intensity that differs by at least 1 nm from that produced by wild-type luciferases.”

The following paragraph replaces the paragraph on page 82, lines 15-24:

“Flavin reductases have been cloned (see, e.g., U.S. Patent No. 5,484,723; See SEQ ID NO. 14 for a representative sequence from this patent). These as well as NAD(P)H can be included in the reaction to regenerate FMNH₂ for reaction with the bacterial luciferase and long chain aldehyde. The flavin reductase catalyzes the reaction of FMN, which is the luciferase reaction, into FMNH₂; thus, if luciferase and the reductase are included in the reaction system, it is possible to maintain the bioluminescence reaction. Namely, since the bacterial luciferase turns over many times, bioluminescence continues as long as a long chain aldehyde is present in the reaction system.”

The following paragraph replaces the paragraph on page 84, lines 16-19:

“As described in detail in the examples below, the frozen tissues were used as a source to isolate nucleic acids encoding *Renilla mulleri* GFP and luciferase (e.g., see SEQ ID NO. 15 and SEQ ID NO. 17, respectively).”

The following paragraph replaces the paragraph on page 87, line 27 to page 88, line 6:

“Thus cloning *Renilla reniformis* GFP clone suggests why many groups may have failed in attempts to clone this gene by traditional methods. An attempt to sequence the entire protein by Edman degradation was difficult from the outset because the GFP was refractory to most attempts at specific proteolysis. Although over 80% of the protein was eventually accurately sequenced, a 30 amino acid region (1190-139 of SEQ ID NO. 27) had not been sequenced (as well as other regions, including amino acids 41-43, 65-71; SEQ ID NO. 27). This 30 amino acid region apparently is degraded by the proteolytic methods used into very small fragments that are difficult to isolate and sequence; proper ordering of sequenced fragments was also difficult.”

The following paragraph replaces the paragraph on page 89, lines 27-31:

“The nucleotide sequence of the cDNA insert of a green fluorescent transformant was determined (e.g., see SEQ ID NO. 15). The 1,079 cDNA insert encodes a 238 amino acid polypeptide that is only 23.5% identical to *A. victoria* GFP. The recombinant protein exhibits excitation and emission spectra similar to those reported for live *Renilla* species.”

The following paragraphs replace the paragraphs on page 90, lines 20-28:

“In preferred embodiments, the DNA fragment encoding a *Renilla* GFP has the sequence of amino acids set forth in SEQ ID NO. 27, encoded by nucleic acid, such as that set forth SEQ ID. NOs. 23-26 and 27.

A DNA molecule encoding a *Renilla* luciferase has the sequence of amino acids set forth in SEQ ID NO. 18. In more preferred embodiments, the DNA fragment encodes the sequence of amino acids encoded by nucleotides 31-963 of the sequence of nucleotides set forth in SEQ ID NO. 17.”

The following paragraphs replace the paragraphs on page 93, lines 14-27:

“In certain embodiments, the recombinant cells may also include heterologous nucleic acid encoding a component of a bioluminescence-generating system, preferably a photoprotein of luciferase. In preferred embodiments, the nucleic acid encoding the bioluminescence-generating system component is isolated from the species *Aequorea*, *Vargula* or

Renilla. In more preferred embodiments, the bioluminescence-generating system component is *Renilla mulleri* luciferase having the amino acid sequence set forth in SEQ ID NO. 18.

Recombinant host cells containing heterologous nucleic acid encoding a *Renilla mulleri* luciferase are also provided. In preferred embodiments, the heterologous nucleic acid encodes the sequence of amino acids as set forth in SEQ ID NO. 18. In more preferred embodiments, the heterologous nucleic acid encodes the sequence of nucleotides set forth in SEQ ID NO. 17.”

The following paragraph replaces the paragraph on page 94, lines 12-20:

“The recombinant cells that contain the heterologous DNA encoding the *Renilla* GFP are produced by transfection with DNA encoding a *Renilla* GFP or luciferase or by introduction of RNA transcripts of DNA encoding a *Renilla* proteins using methods well known to those of skill in the art. The DNA may be introduced as a linear DNA fragment or may be included in an expression vector for stable or transient expression of the encoding DNA. The sequences set forth herein for *Renilla reniformis* GFP are presently preferred (see SEQ ID NOs. 23-25 and 27; see, also SEQ ID NO. 26, which sets forth human optimized codons).”

The following paragraph replaces the paragraph on page 95, line 29 to page 96, line 4:

“In more preferred embodiments, the bioluminescence-generating system component is a *Renilla mulleri* luciferase having the amino acid sequence set forth in SEQ ID NO. 18 or a *Renilla reniformis* luciferase. These compositions can be used in a variety of methods and systems, such as included in conjunction with diagnostic systems for the *in vivo* detection of neoplastic tissues and other tissues, such as those methods described in detail below.”

The following paragraph replaces the paragraph on page 103, lines 8-16:

“Alternatively, an antibody, or F(Ab)₂ antigen-binding fragment thereof of other protein targeting agent may be fused (directly or via a linking peptide) to the luciferase using recombinant DNA technology. For example, the DNA encoding any of the anti-tumor antibodies of

Table 3 may be ligated in the same translational reading frame to DNA encoding any of the above-described luciferases, e.g., SEQ ID NOs. 1-14 and inserted into an expression vector. The DNA encoding the recombinant antibody-luciferase fusion may be introduced into an appropriate host, such as bacteria or yeast, for expression.”

The following paragraph replaces the paragraph on page 117, lines 17-21:

“Kits may be prepared containing the *Renilla reniformis* GFP or the encoding nucleic acid molecules (see, SEQ ID NOs. 23-26) with or without components of a bioluminescence generating system for use in diagnostic and immunoassay methods and with the novelty items, including those described herein.”

The following paragraph replaces the paragraph on page 121, lines 23-28:

“It is also desirable to introduce mutations that alter change. For example, such mutations are those in which R, H and K residues have been replaced with D, such that the hydrophobic and hydrophilic surfaces now each contain 3 mutated residues (SEQ ID NO. 33; Lys to Asp at amino acids 108, 127 and 226, Arg to Asp at amino acids 131 and 199; His to Asp at amino acid 172.”

The following paragraph replaces that paragraph on page 122, lines 18-30:

“Figure 4 exemplifies the site for substitution for inserting fluorophores into the background of *Ptilosarcus*, *Renilla mulleri* and *Renilla reniformis* GFPs. In particular, the 20 amino acid region that lies between two highly conserved pralines with the corresponding 20 amino acid region from any other anthozoan GFP (the underlined regions corresponds to amino acids 56-75 of SEQ ID NO. 27 *Renilla reniformis* GFP; amino acids 59-78 of SEQ ID NO. 16 *Renilla mulleri* GFP; and amino acids 59-78 of SEQ ID NO. 32 for *Ptilosarcus* GFP) is replaced or modified. These 20 residues comprise the bulk of a polypeptide region that threads along the interior of the β -barrel structure that is characteristic of anthozoan GFPs (Wall *et al.* (2000) *Nature Struct. Biol.* 7:1133-1138; Yarbrough *et al.* (2001) *Proc. Natl. Acad. Sci. U.S.A.* 98:462-467); replacement or modification alters spectral properties.”

The following paragraph replaces the paragraph on page 133, lines 3-10:

“Three independent cDNA clones of *Renilla reniformis* GFP were isolated (SEQ ID NOs. 23-25). Each cDNA is full length as judged by identical 5' termini and each encodes an identical protein of 233 amino acids (see SEQ ID NO. 27). Compared to the primary clone (Clone 1), the coding sequence of Clone 2 differs by 4 silent mutations. Clones 2 and 3 also contain small differences in the 5' and 3' untranslated regions of the cDNA. This nucleic acid has been inserted into expression vector, and the encoded protein produced.”